

**Response Under 37 CFR 1.116**

**Expedited Procedure**

**Examining Group 1637**

Application No. 10/584,454

Paper Dated: August 27, 2010

In Reply to USPTO Correspondence of May 27, 2010

Attorney Docket No. 4544-101616

**AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims**

1. (Previously Presented) A set of primers comprising oligonucleotides having nucleotide sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

2. (Previously Presented) A method for detecting and differentiating visceral leishmaniasis (*VL*) and post kala-azar-dermal leishmaniasis (*PKDL*) causing strains of *Leishmania donovani* in a sample, comprising:

- a) isolating DNA from a sample;
- b) amplifying a desired region of the DNA isolated in step (a) using a set of primers comprising oligonucleotides having nucleotide sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, and heat stable DNA polymerase, to obtain amplified fragments;
- c) separating the amplified fragments obtained in step (b) to obtain a separation pattern; and
- d) detecting and differentiating *VL* and *PKDL* causing strains of *Leishmania donovani* by observing differences in the separation pattern of the amplified fragments.

3. (Previously Presented) The method of claim 2, wherein in step (a) the sample is either a clinical sample or culture sample.

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4. (Previously Presented) The method of claim 3, wherein the clinical sample is selected from a group consisting of blood, bone marrow aspirate, bone marrow biopsy, splenic aspirate, splenic biopsy, liver aspirate, liver biopsy, lymph node aspirate, lymph node biopsy, skin scraping, slit biopsy and other tissue materials.

5. (Previously Presented) The method of claim 2 wherein in step (b) the heat stable DNA polymerase is *Taq polymerase*.

6. (Previously Presented) The method of claim 2 wherein in step (b) the amplification is done by polymerase chain reaction.

7. (Previously Presented) The method as claimed in 2 wherein in step (c) the separation is done by gel electrophoresis.

8. (Previously Presented) The method as claimed in 2 wherein in step (d) the detection is by ethidium bromide or other DNA stains.

9. (Previously Presented) A diagnostic kit for detection and differentiation of *VL* and *PKDL* causing strains of the *Leishmania donovani*, comprising a set of primers comprising oligonucleotides having nucleotide sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, reaction buffer, *Taq polymerase*, a DNA marker, positive and negative control samples and an instruction manual.

10. (New) A set of primers comprising a first forward primer having a sequence consisting essentially of SEQ ID NO: 1, a second forward primer have a sequence consisting essentially of SEQ ID NO: 2, a first reverse primer having a sequence consisting essentially of SEQ ID NO: 3, and a second reverse primer having a sequence consisting essentially of SEQ ID NO: 4.

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11. (New) A method for detecting and differentiating visceral leishmaniasis (VL) and post kala-azar-dermal leishmaniasis (PKDL) caused by *Leishmania donovani* comprising:

isolating DNA from a sample;

reacting the isolated DNA with a set of primers comprising a first forward primer having a sequence consisting essentially of SEQ ID NO: 1, a second forward primer having a sequence consisting essentially of SEQ ID NO: 2, a first reverse primer having a sequence consisting essentially of SEQ ID NO: 3, and a second reverse primer having a sequence consisting essentially of SEQ ID NO: 4, thereby forming amplified fragments;

separating the amplified fragments obtained in step (b) to obtain a separation pattern; and

detecting and differentiating *VL* and *PKDL* by observing differences in the separation pattern of the amplified fragments.

12. (New) The method of claim 11, wherein in step (a) the sample is either a clinical sample or culture sample.

13. (New) The method of claim 11, wherein the clinical sample is selected from a group consisting of blood, bone marrow aspirate, bone marrow biopsy, splenic aspirate, splenic biopsy, liver aspirate, liver biopsy, lymph node aspirate, lymph node biopsy, skin scraping, slit biopsy and other tissue materials.

14. (New) The method of claim 11, wherein in step (b) the heat stable DNA polymerase is *Taq polymerase*.

15. (New) The method of claim 11, wherein in step (b) the amplification is done by polymerase chain reaction.

16. (New) The method as claimed in claim 11, wherein in step (c) the separation is done by gel electrophoresis.

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17. (New) The method as claimed in claim 11, wherein in step (d) the detection is by ethidium bromide or other DNA stains.

18. (New) A diagnostic kit for detection and differentiation of visceral leishmaniasis (VL) and post kala-azar-dermal leishmaniasis (PKDL) caused by *Leishmania donovani* comprising: a set of primers comprising a first forward primer having a sequence consisting essentially of SEQ ID NO: 1, a second forward primer have a sequence consisting essentially of SEQ ID NO: 2, a first reverse primer having a sequence consisting essentially of SEQ ID NO: 3, a second reverse primer having a sequence consisting essentially of SEQ ID NO: 4; a reaction buffer; a *Taq* polymerase; a DNA marker; a positive control; and a negative control.